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STUDIES OF SPORE DISSEMINATION OF VENTURIA INAEQUALIS (CKE.) WINT. IN RELATION TO SEASONAL DEVELOPMENT OF APPLE SCAB

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STUDIES OF SPORE DISSEMINATION OF VENTURIA INAEQUALIS (CKE.) WINT. IN RELATION TO SEASONAL DEVELOPMENT OF APPLE SCAB [1]

By C. N. FREY,[2] *formerly Assistant in Plant Pathology, University of Wisconsin,* and G. W. KEITT, *Professor of Plant Pathology, University of Wisconsin*

During the season of 1916 it became apparent to the junior author, who was then beginning work on the control of apple scab,[3] that further studies of certain details of the life history of the causal fungus in relation to the seasonal development of the disease under local conditions were fundamental to the satisfactory application of control measures. A clearer understanding of certain aspects of spore dissemination and infection appeared to be of primary importance. Accordingly, in the spring and summer of 1917, some studies of these questions were undertaken as a minor problem in such time as the writers could devote to it. The advent of the war prevented continuation of this work, and subsequently pressure of duties in another field upon the senior author has prevented earlier publication of the results. However, the junior author and his coworkers have extended these investigations and plan to supplement the following account in a later paper.

A STUDY OF THE SPORE CONTENT OF ORCHARD AIR

METHODS

Of the various methods for the study of spore dissemination described in the literature with which the writers are conversant, none appeared exactly to meet the requirements of their problem. Consequently an attempt was made to devise one.

ELECTRICAL PRECIPITATION

At the suggestion of L. F. Hawley of the Forest Products Laboratory of the United States Department of Agriculture, an attempt was made to adapt to the purposes of the present study the electrical method described by Cottrell [4] for removing suspended particles from gases. Preliminary experiments showed that when spores of *Venturia inaequalis*, *Ustilago zeae*, or certain other organisms were brought within the field of a static machine most of them became negatively charged and passed to the positive pole. A few of the spores tested became positively charged, a small number of each kind always passing to the negative pole.

These observations encouraged the writers to pursue their experiments somewhat further. A glass tube 12 inches long and 1.5 inches in diameter was set up between the poles of the static machine. In each end of this tube was placed a rubber stopper through which ran two glass tubes, each 2 inches long and one-fourth inch in diameter. One of these tubes was attached to a vacuum pump, while the opposite one was left open to permit the access of air. A copper wire was passed through the other two tubes in such manner as to run through the middle of the larger tube and to attach at one end to the negative pole of the static machine. All openings into the larger tube were sealed with wax, with the exception of the two small tubes to permit the ingress and egress of air. Tin foil was lightly wrapped around the outside of the large tube and connected with its inner wall. A copper wire attached to the foil was connected with the positive pole of the static machine. A thin coat of vaseline had previously been rubbed on the inside of the tube to aid in holding the spores as they were precipitated against it. Air currents bearing abundant fungus spores were then passed through the tube while the static machine was in operation. When the current of air was suitably regulated and the static machine delivered a high potential, about 30,000 to 50,000 volts, most of the spores were caught on the first 3 or 4

[1] Received for publication May 24, 1924; issued June, 1925. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

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[3] A comprehensive account of this disease and its causal fungus, *Venturia inaequalis* (Cke.) Wint., with an extensive bibliography, is given in the following citation: WALLACE, E. SCAB DISEASE OF APPLES. N. Y. Cornell Agr. Exp. Sta. Bul. 335, p. 545–624, illus. 1913.

[4] COTTRELL, F. G. THE ELECTRICAL PRECIPITATION OF SUSPENDED PARTICLES. Jour. Indus. and Engin. Chem. 3 : 542–550, illus. 1911.

inches of the tube, being deposited in a thin film on its inner wall. When smut spores were used the tube was quickly coated with a black layer, the darkest zone being situated near the inlet, showing that they were precipitated very promptly upon entering the charged field. When the apparatus was suitably adjusted very few spores passed through the tube. This was determined by passing the outgoing air through a wash bottle containing oil and water and examining the fluid microscopically. Only occasional spores were found.

Preliminary steps were then taken toward developing an electrical precipitation apparatus which might be operated satisfactorily under orchard conditions. However, pressure of work, expense of the electrical equipment necessary, and the fact that very satisfactory results were being obtained by the method described below led to the discontinuance of experiments with the electrical method. The writers believe, however, that this method has possibilities of adaptation to problems involving a study of the spore-content of air. The results obtained appear to be in general agreement with those of Buller (p. 192–195) [5], who has studied the electrical charges on spores from a different point of view.

FILTRATION ON MEMBRANES [6]

Early in the consideration of the problem it occurred to the writers that it might be possible to devise an apparatus which might be run continuously and so constructed that spores could be filtered from the air upon transparent or translucent membranes on which they might be counted under the microscope. Preliminary tests with membranes prepared from cloth, fiber, gelatin, nitrated cellulose, cellulose acetate, and viscose were unsuccessful. The three latter-named materials were not sufficiently porous to allow adequate passage of air. Pure filter paper was tried, and it was found that, by specially treating [7] soft Swedish filter paper, nearly pure cellulose, there was produced a tough, waterproof membrane which proved to be satisfactory. Some special Swedish filter papers and Whatman's hardened filter paper were then investigated and found to be sufficiently waterproof to resist disintegration when wet by rain. The paper, however, should not be so altered by treatment with acid that it loses its porosity and precludes adequate passage of air.

A motor-driven vacuum pump was then set up and a heavy-walled rubber tube was used to connect its intake with a special apparatus for holding the filter paper (fig. 1). It consisted of a thick-walled brass tube (A) on the machined end (B) of which was screwed a very closely fitting brass cap (C) in the middle of which was an aperture 1 inch in diameter. A disc (D) of filter paper of suitable diameter, underlain by a supporting disk of fine silk fabric of similar size, was placed upon the machined end of the metal tube, and the cap screwed tightly over it. In this way it was held firmly in position, the cap making a joint that was air-tight, while a circular area 1 inch in diameter was exposed for purposes of filtration. A perforated rubber stopper (E) connected the device with the suction tube by means of a glass tube. Numerous tests of the capacity of the machine with different filters of the type used throughout the experiments showed that approximately 1,200 liters of air passed through the apparatus in an hour.

The membranes were removed at intervals as circumstances warranted. As soon as a membrane was removed it was placed in a small Esmarck dish and treated with a small amount of glycerine to make sure that the spores remained in place. In each test a membrane similar to the one used for filtration was placed beside the latter, collected with it, and studied microscopically, as a control against chance accumulation of air-borne ascospores. Studies of these, however, showed so few spores that it seemed unnecessary to make any correction in the results for them. The number of spores caught on the membrane was determined by direct counts under the microscope. The olivaceous color and characteristic form and size of the spores made them easily observed without staining. If the number was

[5] BULLER, A. H. R. RESEARCHES ON FUNGI. v. 1, illus. London, New York, etc. 1909.

[6] In later studies the junior author and his coworkers have developed another filtration technique which is being used in their further work. Orchard air is drawn by means of a motor-driven suction apparatus through a suitably arranged filter of nitrocellulose. Thence it passes through a gas meter which records the volume. At the end of the run, the nitrocullulose filter, bearing the spores caught, is dissolved in a suitable glass container in a mixture of alcohol and ether and allowed to evaporate to a gel. The ascospores settle to the bottom and their number is computed on the basis of microscopic counts. This is an adaptation of a technique developed by Pasteur. It will be described in more detail in a later paper.

[7] The filter paper was dipped for about one-half minute in nitric acid, sp. gr. 1.42; 2, transferred for brief period to sulphuric acid (equal volumes of 1.84 sp. gr. sulphuric acid and water), and washed in water and then in a weak solution of ammonia.

small or nil the entire surface of the exposed area of the membrane was observed. This was accomplished and duplication of observation avoided by the use of a mechanical stage and a micrometer. If spores were numerous, counts were made of at least 30 fields, chosen at random but without duplication from all parts of the filter, and the total number was computed on the basis of this average. The results thus obtained agreed closely with those from counts over the entire exposed area. A Leitz microscope was used, with a No. 4 eyepiece, a No. 6 objective, and a tube length of 170 mm.

FIELD EXPERIMENTS

During late April and early May preliminary observations were made to determine the time at which ascospores would be sufficiently mature for natural discharge. Over-wintered apple leaves bearing numerous perithecia of *Venturia inaequalis* were brought into the laboratory daily, thoroughly moistened, and placed in moist chambers in such position that one could ascertain by miscroscopic examination of the glass surface below them whether or not ascospores had been discharged. No discharge from such material was observed until May 7.

On May 6, just as the cluster buds of the apple were beginning to open, the apparatus was installed in the Turville[8] orchard, about 1½ miles south of Madison. This orchard, consisting of about 50 large trees planted in four rows running east and west, was in sod, and the ground under and about the trees was abundantly littered with over-wintered leaves which bore perithecia of *Venturia inaequalis* in great profusion. The apparatus was placed under the outer branches of a Fameuse tree which stood in the southernmost row, second from the eastern end. The membrane was placed at elevations varying from 1½ to 3 feet above the ground, and was protected from rain by a small roof-like shelter which was raised sufficiently to permit free passage of air over the filter. The machine was run continuously, except for brief stops for changing membranes and caring for the motor, until June 19, and subsequently at intervals. The results are summarized in Table I in correlation with hourly records of rainfall. Hourly records of temperature and wind velocity show valuable correlations but are not included in the table because of limitations of space.

The field and laboratory observations indicate that on May 6, when the field filtration tests were started, the ascospores were just approaching maturity. Apparently no discharge had occurred prior to this time. During the dry period from May 6 to 18 no Venturia spores were caught. On May 19, rain fell from about 2 to 5

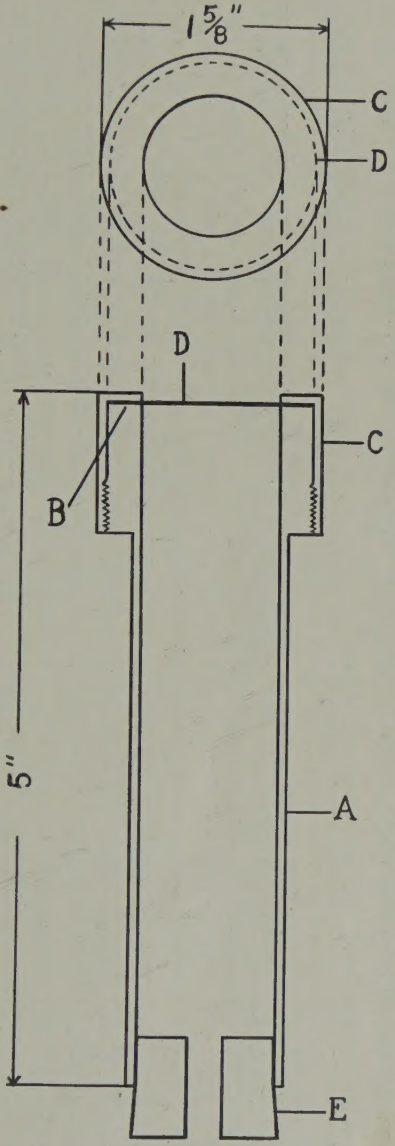
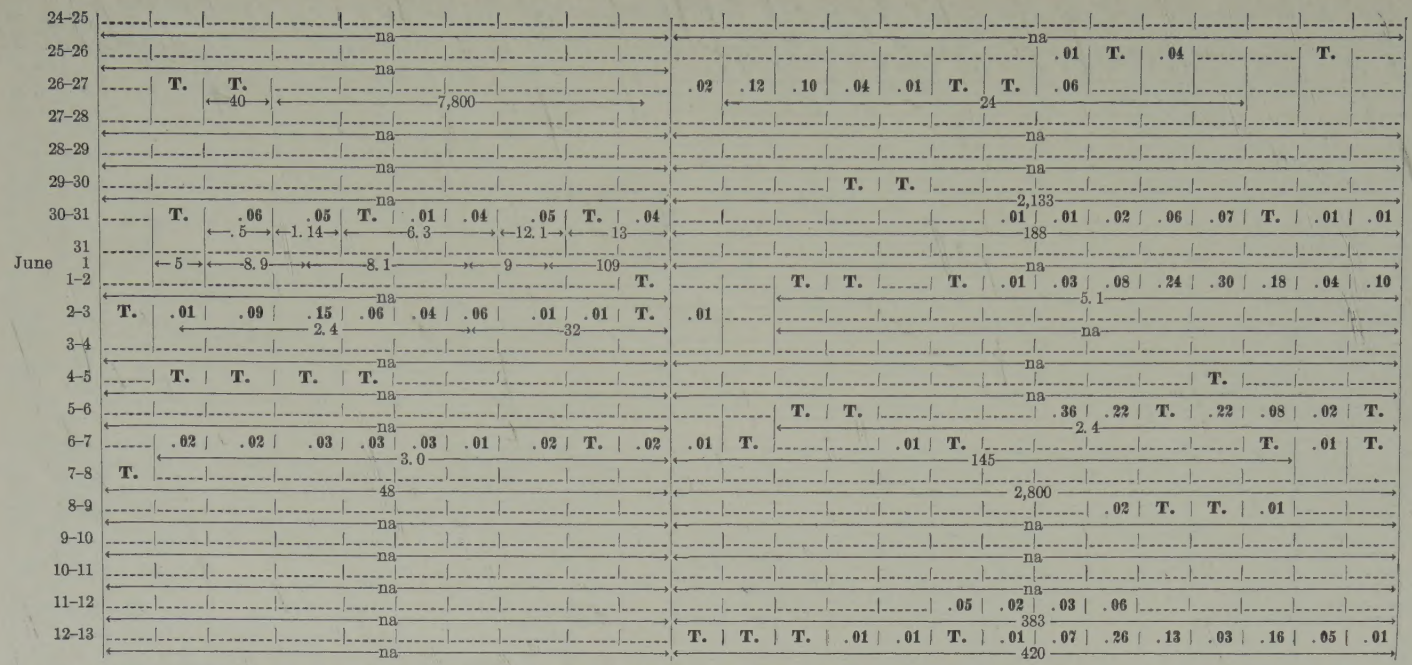


FIG. 1.—Device used for filtering spores from the air

[8] Grateful acknowledgments are made to Thomas and Wm. D. Turville for their kindness in making their orchard available for this work.

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*

Dates	Records by hours[a]																								Total rainfall (inches)
	A. M.				P. M.												A. M.								
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
May 5–6																									0
6–7	na										na														0
7–8	na										na						T.					T.			T.
8–9	na										na														0
9–10	na										na														0
10–11	na										na														0
11–12	na										na														0
12–13	na										na														0
13–14	na										na														0
14–15	na										na														0
15–16	na										na														0
16–17	na										na														7
17–18	na										na														0
18–19	na										na						T.	.54		.23					.77
19–20	na										30											T.			T.
20–21	89				204						840			T.	.11	T.	T.	T.	.08	.02	.01	.03	.05	.03	.33
21–22	.01	.09	.23	.17	.16	.03	T.	T.	na		(?)[b]						.01	.05	T.	.03	.05	.05	.07	.07	1.06
22–23	.07	.04	.05	.03	.01	4					23													.04	.20
23–24	na										109						na								0



[a] In the space representing each hour, the upper line (the line of the date) is devoted to the rainfall record, expressed in inches. In order to make the rain periods stand out more sharply, zeros are omitted and bold-faced type is used. "T" indicates that only traces of rain fell.

The lower line gives the ascospore discharge record. The arrows indicate the periods during which the filter was operated. The numbers between the arrows represent the average number of liters of air filtered for each ascospore caught. *"na" indicates that no ascospores were caught during the period shown*, though air was filtered at the standard rate of approximately 1,200 liters per hour.

The data on rainfall are taken from the records of the Madison station of the U. S. Weather Bureau, which is situated on the top of a four-story building about 1½ miles from the Turville orchard. These data are therefore not to be interpreted as representing more than a close approximation of the conditions which obtained in the orchard. Inches of rainfall on the dates omitted from the table follow: June 20–21, 0.03; 22–23, 1.24; 23–24, 0.01; 24–25, 0.01; 25–26, 0.53; 27–28, 0.45; 28–29, 0.60; 29–30, 0.10; July 5–6, 0.03; 6–7, 0.07; 10–11, 0.03; 13–14, 0.15.

[b] The filter was so covered by sleet that no reliable record could be obtained.

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*—Continued

Dates	Records by hours																								Total rainfall (inches
	A. M.				P. M.												A. M.								
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
June13–14	T.	T.	.02								T.	.01													.03
											137														
14–15			T.	T.	T.			T.	T.	T.	T.	T.				.01									.01
											1,000														
15–16																									0
	na										na														
16–17														T.	T.										T.
	na										na														
17–18																									0
	na										na														
18–19																									0
	na										na														
19–20																									0
	na										na														
30																	.01	.08		.51	.02				62
July 1	na										30														
1–2																									0
	na										na														
2–3																									0
	na										na														
3–4																									0
	na										na														
11–12		.14			T.		T.			T.	.01	T.													.15
											33														
12–13																									0
	na																								
17–18								.07	.01			.02	T.												.10
											56														
18–19											.07	T.													.07
											na														
19–20																									0
	na										na														
21–22																		.08	1.28						1.36
											na														

a. m., and the first discharge of the season was recorded by the apparatus. Ascospores were caught at the average rate of 1 ascospore to 30 liters for the 3-hour period covered. From 8.30 to 10.30 a. m., while the leaves were still wet, the average rate of catch was 1 ascospore to 89 liters. From 10.30 a. m. to 6 p. m. it was 1 to 204 liters; and from 6 p. m., May 19, to 8 a. m., May 20, 1 to 840 liters. No spores were caught from 8 a. m. to 6 p. m., May 20, by which time the leaves had thoroughly dried.

During the night of May 20–21 rain or sleet fell almost continuously and rather high winds prevailed. Unfortunately, in spite of the shelter which had been erected to protect the membrane from weather, it was so washed by rain and sleet that no trustworthy record could be taken. In the morning, however, the winds became less violent. The rain continued from 9 a. m., when a new membrane was put on, until about 4 p. m. From 9 a. m. to 5.30 p. m. ascospores were caught at the average rate of 1 ascospore to 0.4 liters. This proved to be the heaviest discharge recorded during the experiments. The wind velocity for the period of this run varied from 14 to 23 miles an hour.

Rain began to fall about 11 p. m. May 21 and continued until about 1 p. m. May 22. From 7 p. m., May 21, to 7 a. m., May 22, ascospores were caught at the average rate of 1 ascospore to 23 liters. The wind velocity for the period during which this rain fell varied from 21 to 34 miles an hour (NE. to NW.). No record is available for the daylight hours of May 22. The machine ran from 6 p. m., May 22, to 8 a. m., May 23, and recorded an average catch of 1 ascospore to 109 liters. Apparently the leaves were sufficiently moist from the morning's rain to continue this light discharge. Following thorough drying the next morning, discharge ceased and no spores were caught until the next rain.

Light showers fell between 1 and 7 a. m., May 26, but no record for this period is available. From 10 to 11 a. m., however, during traces of rainfall, the machine was run, and ascospores were caught at the rate of 1 ascospore to 40 liters. From 11 a. m. to 5.30 p. m., with no additional rainfall, the catch was only 1 to 7,800 liters.

Showers fell during the night of May 26–27, and spores were caught at the rate of 1 ascospore to 24 liters. The wind velocity during this rain period varied from 10 to 22 miles.

No more ascospores were registered by the machine until the night of May 29–30, when, following traces of rain, they were caught at the rate of 1 ascospore to 2,133 liters. On May 30, however, an excellent opportunity for a fractional study of a discharge period presented itself. From 9 to 10 a. m. a trace of rain fell, followed by a fairly heavy rain from 10 to 12, a trace from 12 to 1, and almost continuous rain throughout the remainder of the afternoon. The wind velocity from 10 a. m. to 6 p. m. varied from 13 to 23 miles an hour. From 10 to 11 a. m. ascopores were caught at the average rate of 1 ascospore to 0.5 liters; from 11 to 12, 1 to 1.14; from 12 to 3, 1 to 6.3; from 3 to 4, 1 to 12.1; from 4 to 6, 1 to 13. From midnight until 8 a. m., May 31, it rained almost continuously. However, from 6 p. m., May 30, to 8 a. m., May 31, the average rate of catch was only 1 ascospore to 188 liters. The old leaves on the ground remained wet in the tall grass of the orchard until about 3:30 p. m., May 31. By this time the wind, which had freshened about 3 p. m., had begun to dry them perceptibly. This is very well reflected in the ascospore content of the air. From 9 to 10 a. m., May 31, spores were caught at the rate of 1 ascospore to 5 liters of air; from 10 to 11.30, 1 to 8.9; from 11.30 to 2.30, 1 to 8.1; from 2.30 to 3.40, 1 to 9; from 3.40 to 6, 1 to 109. It appears that, at the beginning of this rain period, there was an abundance of mature ascospores ready for discharge. During the first 2 hours (10 to 12 a. m., May 30) the rate of discharge was very high. Subsequently, however, it dwindled rapidly, and relatively few mature spores appear to have been available for discharge during the rain of the following night. The rate of discharge increased very markedly on the morning of May 31, probably due to the maturing of asci during the current moist period.

No further discharge was recorded until the rain of the night of June 1–2, when ascospores were caught at the rate of 1 ascospore to 5.1 liters (8 p. m. to 8 a. m.). From 9.30 a. m. to 2.30 p. m., June 2, it rained continuously, and ascospores were caught at the rate of 1 ascospore to 2.4 liters. From 2.30 to 6 p. m., although showers continued, the rate of catch fell to 1 to 32 liters. During the following night, no spores were caught. In this instance, the period of rapid discharge was considerably more prolonged than was the case in the preceding rain.

No further discharge was recorded until the rain of the night, June 5–6, when the average rate of catch from 8 p. m. to 8 a. m. was 1 ascospore to 2.4 liters. The rain continued through-

out the day of June 6, the catch from 9 a. m. to 6 p. m. being at the rate of 1 to 3 liters. During the following night, although there were slight showers, the rate fell to 1 ascospore to 145 liters. In this, as in the preceding instance, the period of rapid discharge was fairly prolonged, probably extending over about 15 hours. It appears likely that the more extended periods of these later heavy discharges, as compared with that of May 31, are to be attributed to a more generally advanced stage of maturity of asci and possibly to more rapid ripening.

Minor discharges occurred during the showers of June 7, 12, 13, 14, and 15. Although fairly heavy rains fell on June 12 and 13, the discharges were very light. It appears that the heavy discharges on and prior to June 7 went far toward exhausting the supply of ascospores. The failure to catch any spores whatever during the rainfall of 0.03 inch of June 9 is not understood. It is possible that there was less precipitation in the orchard than at the weather station. It is conceivable, on the other hand, that the supply of ripening asci was so nearly exhausted that no significant numbers were in condition to discharge at this time.

No spores were caught during the dry period from June 16 to 20.

Due to the absence of the senior author, no record was taken for the period of June 21 to 29.

Rain fell in the latter part of the night of June 30–July 1. From 6 p. m. to 8 a. m., the average rate of catch was 1 ascospore to 30 liters. When it is considered that most of the discharge of this period evidently occurred from about midnight to 5 or 6 a. m., it is apparent that this represents a fairly heavy discharge for so late a date.

During the dry period, July 2 to 4, no ascospores were caught.

The next run was made on the night July 11–12, when light showers fell from about 6 to 8 p. m. The catch was at the rate of 1 ascospore to 33 liters. From 8.30 a. m. to 6 p. m. July 12, there was no rain, and no spores were caught.

The next run was made on the night of July 17–18. Light showers fell between 7 and 9 p. m. From 6 p. m. to 8 a. m. the rate of catch was 1 ascospore to 56 liters.

Showers fell from 6 to 8 p. m., July 18. No ascospores were caught in a run from 6 p. m., July 18, to 8 a. m., July 19. During the following 24 hours, no rain fell and no spores were caught.

From 2 to 4 a. m., July 22, 1.36 inches of rain fell. From 6 p. m., July 21, to 8 a. m., July 22, no ascospores were caught. It appears that the season's supply was exhausted at this time.

From the results of these experiments it is readily apparent that the presence of an adequate amount of water is a primary requisite for the ejection of ascospores of *Venturia inaequalis*. Under the conditions of the tests, dew alone was not sufficient to occasion discharges of any considerable consequence. It is apparent, further, that rain water is very important, not only in relation to the discharge of ascospores, but for their maturation as well. This latter point has been more clearly established in later studies by the junior author and his coworkers and will be discussed in a later paper.

The records of May 31, June 3, and June 6–7 show that when ascospores in abundance are in condition to be ejected, heavy discharges occur at the beginning of a rain period and continue during continuous rain as long as the supply of ripe asci lasts. The periods of heavy discharge varied from several hours on May 31 to about 15 hours on June 3 and June 6–7.

The maximal average concentration of spores for a single run of the machine, 1 ascospore to 0.4 liters, was obtained between 9 a. m. and 5.30 p. m., May 21. When one considers that only traces of rain fell after 2 p. m., it appears likely that, at the period of maximal discharge, the concentration of spores may have been considerably greater than the average for the entire run. In order to gain a still more concrete conception of such concentrations of spores it seems to be of value to compute on the basis of the records just mentioned the number of ascospores which would have passed in the 8½-hour period studied through an imaginary orifice 10 cm. square interposed 3 feet above ground perpendicular to the wind direction. With the average wind velocity for this period of 18.5 miles an hour and the average ascospore content of the air 3 feet above ground of 1 ascospore to 0.4 liters, as shown by the records, it appears that over 6,300,000 ascospores would have passed through the orifice postulated.

In considering these records of ascospore discharge in relation to the seasonal development of the host plant and to control measures it would appear that they are in accord with the widely prevalent idea that the first ascospore discharges of *Venturia*

inaequalis are to be expected at about the time the fruit buds separate in the clusters just prior to the blooming period. Subsequent studies, however, have shown that, in the vicinities of Madison and Sturgeon Bay, Wis., ascospore discharge ordinarily begins at a much earlier stage of bud development and that the lateness of the initial discharge of 1917 may be attributed largely to the paucity of rainfall in early April and the first 18 days of May. It should be noted that even in 1917 it was shown by wetting leaves freshly collected from the orchard that asci were capable of discharging spores as early as May 7.

Although the air filtration experiments were planned primarily for the study of ascospore dissemination a careful watch was kept for conidia. After June 2, when the first scab lesions were observed, conidia began to appear in small numbers on the membrane in rainy periods, particularly when the rain was accompanied by high winds. They were never caught, however, except under these conditions. These facts led the writers to make certain observations upon the conditions which favor dissemination of conidia.

Apples leaves bearing abundantly sporulating scab lesions were placed in a glass tube through which a strong current of air was driven. A glass slide smeared with glycerine was held opposite the outlet of the tube in position to catch air-borne spores. Microscopic examinations following numerous repetitions of this test revealed only very small numbers of spores on the slides. Similar experiments with lesions on apple fruit gave like results. However, when these same lesions were treated with a fine mist of water applied by means of an atomizer, the droplets which accumulated contained conidia in great numbers. Similarly, when sections were cut from lesions with a dry razor and observed under the microscope, the addition of a droplet of water led to swelling of the conidiophores and immediate detachment of conidia. Furthermore, drippings collected during rains from scabby apple trees have shown an abundant content of conidia of *Venturia inaequalis*. These results, in conjunction with those from the air filtration experiments, indicate that no important dissemination of conidia is to be expected in the absence of water, though undoubtedly some spores are dislodged by wind-whipping of leaves, fruit, or branches, by contact with wind-blown particles, and in other minor ways. It appears, therefore, that the important agency for dissemination of these conidia is meteoric water moving under the influence of wind and gravitation.

GERMINATION TESTS

Ascospores.—Germination tests with ascospores were made at frequent intervals throughout the period in which their natural discharge was observed in the field (May 18 to July 18). Fragments of leaves bearing perithecia were moistened and placed in such position that the spores would be discharged upon droplets of sterile distilled water on clean sterile glass slides in moist chambers or on plates of 2 per cent agar in water. The drops or plates were then placed in incubators at 18° to 25°C. In all cases naturally discharged ascospores showed a high percentage of vigorous germination, usually approximating 100 per cent. Germination tests of ascospores caught on the membranes gave like results. Some experiments conducted at constant temperatures ranging from 2° to 26° C. showed germination at all the temperatures tried, the optimum for germination and growth under these conditions being between 14° and 20° C.

Conidia.—Germination tests of conidia from fruit and leaf lesions were made at frequent intervals from their first appearance until late fall. The same media and incubators used for ascospore germination were employed. Abundant viable conidia could be found at any time during these experiments, but the percentage and vigor of germination varied greatly in individual tests. This is not surprising in view of the fact that mature conidia are much less protected than are ascospores and not so uniformly removed from the parent fungus when rain follows their maturity. Consequently, a sample of conidia, even when secured by touching a droplet of water borne in a wire loop to the sporulating surface of a scab lesion, is likely to be less uniform than a sample of naturally discharged ascospores.

SEASONAL DEVELOPMENT OF THE HOST PLANT

In relation to production and dissemination of spores and the seasonal development of the disease, the following brief notes on the seasonal development of the host plant are pertinent:

The unfolding of both vegetative and fruit buds was unusually late and slow in 1917. On May 7 the pink of the

petals of many fruit buds was evident, and by May 18 nearly all of the blossoms were open. On May 22 the largest leaves were about 2½ inches long, and petals were falling. The last leaves to develop appeared from about May 19 to 24, some variation occurring with varieties and individual trees. Most of the petals were off by May 25, and little leaf expansion occurred after June 2, when the first conidia of the season were observed. There was practically no leaf expansion after June 8 (except on "water sprouts").

SEASONAL DEVELOPMENT OF THE DISEASE

The seasonal development of the disease was studied by means of a series of bagging experiments, supplemented by daily observations in the orchard. No spray was used in this orchard. Each day from May 7 to June 19, six branches on at least two varieties were inclosed in large manila paper bags which were securely fastened in place with strong twine. In this way comparative records were kept of the development of the disease on branches which had been exposed to different infection periods. Many of the bags were broken [9] during storms, necessitating discarding a considerable number of branches from the experiment. Enough were left, however, to give some valuable data, a brief account of which follows.

None of the branches which were bagged before May 19 and on which the bags remained unbroken during the storms of May 30 and June 2 showed any evidence of infection throughout the period of observation, which ended on August 24. All branches bagged after May 22 developed infection, and with the exception of the last two leaves formed, which in some cases were put out after May 22, developed about as many leaf lesions as those which were continuously exposed to infection. Leaves of branches bagged after the rain on June 2 were infected in the same manner and to approximately the same extent as those of similar unbagged branches. This is of especial interest in view of the heavy ascospore discharge and favorable infection period of June 6–7.

The first leaf infection of the season was noted on June 2. This would allow a 14-day incubation period after the rain of May 19. This agrees with the results of field inoculation studies made in early spring in later years, in which the periods of incubation for leaf infection have commonly varied from 13 to 17 days. The records on ascospore dissemination and seasonal development of the disease are, therefore, in accord in placing the first ascospore discharge and infection of the season in the rainy period of May 19 to 22. The fact that no infection of significance occurred on leaves of branches which were bagged during the period May 19 to June 2 and subsequently exposed indicates that the leaves had become highly resistant to infection by the scab fungus by the latter date.

A striking suggestion of the variation in the susceptibility of leaves in relation to their stage of development was found in the number, distribution, and incubation periods of leaf lesions induced by natural infection. On June 2, when the first scab lesions of the season were observed, leaf expansion was almost finished. Lesions were clearly visible, in most cases, only on the upper surfaces of the apical leaves (for convenience called leaves No. 1) of shoots. Occasionally, early evidences of infection were apparent on the next leaf back, and within a day or two leaves No. 2 showed infection in about the same degree as No. 1. The development on leaves No. 3 and No. 4 was visible by June 8, while by June 12 to 15 small lesions were evident on many leaves No. 5 and No. 6 and rarely on No. 7. In late July and August the older leaves frequently showed traces of fungous growth on their lower surfaces but no definite lesions. The earlier infection thus appeared on the younger leaves and usually on the upper surfaces after relatively short incubation periods, while the latest infections to become evident appeared after prolonged incubation on the lower surfaces of the older leaves. Later field observations and field and greenhouse inoculation studies have confirmed the general outlines of these observations. They have shown, however, that there is a considerable range in the degree of these variations under different conditions. Under some conditions, for instance, only one or two leaves of a shoot will become infected from a given inoculation, while under others infection may develop on a half dozen or more.

The seasonal development of the disease on the fruit was much more difficult to follow in detail than on the leaves because of shedding induced by insect injury and other causes. The

[9] In later seasons the substitution of bags made of parchment paper obviated this difficulty.

first observation of fruit infection was made on June 8, when lesions were visible on the young fruits, calyx lobes, and pedicels. Daily field records showed that lesions continued to appear on the fruit throughout the period of observation, which ended on August 24. The results from the bagging work showed that the first fruit infection occurred during the rainy period of May 19 to 23. The most abundant infection and the largest lesions developed in the early part of the season. The fact that the later lesions are smaller suggests that the fruit, like the leaf, may develop resistance with age. Such resistance, however, appears to be developed in a much smaller degree, since fruit infection has been shown to appear late in the season or even in storage.

INOCULATION EXPERIMENTS

The life history studies were supplemented by some inoculation work in the orchard. Metal boxes, 28 by 12 by 10 inches, with tightly fitting, felt-lined covers, were constructed as infection chambers. A slit at one end of each box permitted a branch to enter and a tripod served as an adjustable support. The box was placed in position about the inoculated or control branch, the cover put in place, and the orifice through which the branch entered closed with plastic clay. A metal shelter about 4 inches above the box shaded it from the sun. When necessary the box was cooled by water.

Three types of shoots were selected for inoculation: (1) Those which had been bagged continuously since May 18 and were therefore free from infection, (2) those which had been exposed to infection throughout their development, and (3) those which developed during July ("water sprouts") and consequently bore young leaves. The inocula consisted of sterile-water suspensions of conidia from naturally infected leaves and from oatmeal-agar cultures. The experiments were performed in July and early August.

The chief results of these experiments may be summarized briefly. Thirty-six trials on disease-free shoots which had been bagged prior to May 18, and which bore no young leaves, gave no infection. In similar trials on shoots which had been exposed continuously to infection, there was no evidence that additional infection was induced. However, on branches which bore young leaves, abundant infection was induced on the young leaves provided the temperature was kept from running too high. While the exact maximum temperature for infection was not determined it appeared to be not much higher than 25° C. The maximum age of susceptible leaves is of course variable with conditions. In these tests it usually ranged between 12 and 15 days. These results are therefore in accord with those obtained in the life history and seasonal development studies.

SUMMARY

An effort was made to devise a satisfactory apparatus for determining the spore content of orchard air. Preliminary experiments were made with an electrical device. The results were promising, but the greater economy and simplicity of a mechanical filter led to its adoption for this work. It is believed, however, that the electrical method has potentialities of adaptation to problems of this type.

By wetting leaves freshly collected from the orchard it was shown that, in the vicinity of Madison, Wis., asci were capable of discharge under favorable conditions on May 7, 1917. Because of dry weather, however, the first natural discharge was delayed until May 19. Following this date ascospore discharges occurred during rains throughout the spring and early summer, the last recorded discharge occurring on July 18. The very heavy discharges, however, were limited to the period May 19 to June 7. After this time the discharges were relatively small.

The maximal concentration of ascospores of *Venturia inaequalis* in the orchard air was observed on May 21, when the average rate of catch for a period of 8½ hours was 1 ascospore to 0.4 liters of air. The wind velocity for this period varied from 14 to 23 miles an hour. The filter was 3 feet above the ground.

It was found that, when the asci were in condition to eject their spores, the presence of an adequate supply of water was the most important requisite for their discharge. In the experiments dew was not sufficient to induce discharges of consequence.

In cases where abundant asci were in condition to eject their spores in the presence of water, heavy discharge started soon after rain began and continued with continuous rain as long as the supply of ripe asci lasted. These periods of very heavy discharge lasted from 3 to 15 hours. Undoubtedly the duration of such periods varies widely with conditions.

Conidia of *Venturia inaequalis* were found in the air only during rain periods, and particularly when rain was accompanied by strong wind. Experiments showed that these conidia are very resistant to detachment from their conidiophores when dry, but quickly become detached in the presence of water. They are, therefore, disseminated chiefly in meteoric water acting under the influence of wind and gravitation.

Germination tests showed that practically all naturally discharged ascospores were vigorously viable. Conidia germinated with much less regularity. At no time after their appearance in the spring, however, was it difficult to find viable conidia in abundance.

The seasonal development of the host plant was unusually slow in the spring of 1917. In the Turville orchard most of the blossoms were open by May 18, and petal-fall was about complete on May 25. The last leaves to develop appeared from about May 19 to 24, and very little leaf expansion occurred after June 8.

Bagging experiments and orchard observations showed that most of the leaf infection of the season occurred during the period of May 19 to June 2, during which time all but one of the major ascospore discharges of the season occurred. The first observation of leaf lesions was made on June 2, 14 days after the first period of ascospore discharge. As the leaves approached maturity they became highly resistant to infection by the scab fungus.

Fruit infections were observed on June 8 and continued to appear throughout the period of observation, which ended on August 24. The records indicate that the first fruit infection occurred during the rainy period of May 19 to 23.

Successful leaf inoculation experiments were conducted in the orchard upon branches inclosed in specially constructed moist chambers. Infection from conidia applied in suspension in water was secured at will upon young leaves if fairly low temperatures (maximum at which infection occurred somewhat above 25°C.) were maintained. Old leaves were highly resistant to infection by *Venturia inaequalis*.